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Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19

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Abstract

Pathogenic viruses represent one of the greatest threats to human well-being. As evidenced by the COVID-19 global pandemic, however, halting the spread of highly contagious diseases is notoriously difficult. Successful control strategies therefore have to rely on effective surveillance. Here, we describe how monitoring wastewater from urban areas can be used to detect the arrival and subsequent decline of pathogens, such as SARS-CoV-2. As the amount of virus shed in faeces and urine varies largely from person to person, it is very difficult to quantitatively determine the number of people who are infected in the population. More research on the surveillance of viruses in wastewater using accurate and validated methods, as well as subsequent risk analysis and modelling is paramount in understanding the dynamics of viral outbreaks.

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Keywords

COVID-19, SARS-CoV-2, Coronavirus, Wastewater-based epidemiology, Virus surveillance, Risk assessment, Public health.

Introduction

Recent decades have seen a marked rise in the number of novel and emerging human pathogenic viruses. This has resulted in a range of globally significant outbreaks and epidemics and a major loss of life. Examples include the SARS coronavirus (SARS-CoV-1) epidemic in 2003 with more than 8000 cases in 29 countries, the H1N1 influenza pandemic in 2009–2010 with 60 million cases in 214 countries, and the MERS coronavirus epidemic in 2012–2015 with approx. 2500 cases in 27 countries (www.who.int). In December 2019, an outbreak related to a novel coronavirus (SARS-CoV-2) was reported in China which has been rapidly spreading globally with more than 6 million confirmed cases and more than 376,000 deaths by 2nd June, 2020 [1].

Owing to the often high infectivity and rapid transmission of viruses, individual screening in clinical settings is often challenging. In addition, cases with mild or no symptoms are often overlooked, and hence epidemiological models and assessments of disease prevalence may be inaccurate. There is therefore a greater need to understand the spread of viral diseases at a community level which would provide information for the timely mitigation of outbreaks.

Municipal wastewater harbours a great variety of pathogenic viruses [2]. Extensive research has been undertaken on the persistence of human enteric viruses (e.g. noroviruses, enteroviruses, adenoviruses, rotaviruses, hepatitis A/E viruses), transmitted via the faecal-oral route, in wastewater and in the aquatic environment [3]. Enveloped viruses (e.g. coronaviruses), which rapidly inactivate without a host, have also been found in wastewater [3]. Temporal changes in viral concentrations in wastewater can therefore indicate the presence or absence of a virus, related outbreaks in the population, and their effect on public health. Hence, domestic wastewater monitoring may be an important tool to assess and mitigate viral outbreaks in a community. In this review, we aim to critically assess the recent efforts on using wastewater surveillance to represent public health, with a focus on SARS-CoV-2 surveillance.

The current toolbox for wastewater viral monitoring

Wastewater concentration for virus detection

For the sensitive detection of viruses in wastewater, samples are often concentrated before quantification. Many different approaches are commonly used, as recently reviewed [4,5]. For the surveillance of SARS-

CoV-2, wastewater samples are often centrifuged or filtered to eliminate debris, followed by electronegative membrane filtration [6], ultrafiltration [6—11] or polyethylene glycol precipitation [8,9,12,13], aluminium flocculation [14,15] or ultracentrifugation [16,17] enabling 20x-800x concentration. Sludge samples were either subject to RNA extraction directly [18], or viruses were eluted and PEG precipitated from the matrix [11]. Most concentration methods are inexpensive and easy to set-up, however, they may be time-consuming and difficult to perform with high sample throughput, especially when high turbidity samples are processed. The main disadvantage of these methods is the co-concentration of organic compounds (e.g. humic substances), which often interfere with downstream virus detection or *in vitro* studies. Furthermore, concentration efficiency may vary among different samples, however, it has only been assessed in two studies aiming to detect SARS-CoV-2 in wastewater [7,15], suggesting 3—50% viral recoveries (Table 1). Therefore, appropriate process controls, for example, viruses of the same family or genus should be added to the sample to estimate viral recoveries [15]. Alternatively, the concentration of a viral indicator, which is present in wastewater at high concentrations (e.g. gut-associated phages), can be compared between unprocessed and processed samples to assess concentration efficiency [7].

Amplification-based viral quantification

The most widely used methods for quantification of DNA and RNA viruses in wastewater are quantitative PCR (qPCR) and quantitative reverse transcription PCR (qRT-PCR), respectively [4,19]. These methods detect a small segment of the viral genome, enabling rapid, sensitive and accurate strain-level detection of up to five targets in one assay [20]. Several qRT-PCR assays have been designed for the detection of SARS-CoV-2 [21—24], which are suitable for wastewater monitoring [6,7,12,16]; however, the performance of the different assays may vary. Substantial differences in viral detection rates were observed when different primer/probes were used for quantification. For example, the 'N2' assay did not detect SARS-CoV-2 in wastewater samples which were positive for the 'N1' and 'N3' genes [7]; hence the use of multiple primer/probe sets is recommended. A limitation of qPCR-based approaches is that the reverse transcription and polymerase enzymes are often inhibited by organic co-contaminants, which are concentrated and extracted together with the targets.

Recently, digital PCR—based approaches have also been used for viral detection in environmental samples [19]. These methods enable the absolute quantification of the targets and are less sensitive to inhibition, however more expensive than qPCR-based assays. Other emerging technologies, including isothermal amplification and biosensors, are also suitable for viral RNA/DNA detection and quantification in environmental samples, providing results within an hour [19]. Simple and affordable platforms (e.g. paper-based microfluidics devices) also have great potential for rapid, on-site viral detection in wastewater [25], however, these assays are not as sensitive yet as traditional, PCR-based methods and have not been rigorously tested in the field [26].

Culture-based analysis of viral infectivity

Most human viruses are difficult to maintain *in vitro* and their culturing requires trained staff and specialized equipment. Hence, infectivity assays are rarely performed on wastewater samples. To date, the infectivity of SARS-CoV-2 in wastewater has not been assessed, even though culturable viral particles have been detected in the faeces and urine of infected individuals [27,28]. These studies typically use Vero E6 cells to culture the SARS-CoV-2, and a similar approach may be suitable for the widespread screening of wastewater samples. Nonetheless, to investigate the temporal changes of viral infections in the community, molecular detection of viral genomes is sufficient.

Viromics and sequencing

Viral metagenomics of wastewater has been widely used to monitor the prevalence of multiple pathogens and could be used as an early warning system for the detection of outbreaks of novel viral pathogens [29,30]. For example, a high-throughput sequencing approach was used as an alternative to q(RT-)PCR to explore the diversity of enterovirus D, hepatitis A and hepatitis E viruses [31] and mastadenovirus [32] in wastewater to assess the viral strains circulating in local populations of France and Australia, respectively. It may also be useful to monitor other respiratory viruses (e.g. influenza) alongside SARS-CoV-2, given the uncertainties about whether coinfection affects the outcome of COVID-19 cases. Untargeted sequencing applied during outbreaks can monitor genetic drift that might affect the detection efficacy of amplification primers used in both sequencing studies and in qPCR-based diagnostic tests. For example, complete genomes of norovirus have been recovered from wastewater containing mismatches in primer regions which would not amplify in qRT-PCR assays [2]. So far, three studies performed sequencing of SARS-CoV-2 (q)PCR products derived from wastewater to verify the presence and potential origin of SARS-CoV-2 [6,10,33]. Untargeted sequencing has not been used to investigate SARS-CoV-2 strains in wastewater.

Virus surveillance in wastewater

Most studies on virus surveillance in wastewater have focused on the prevalence of human enteric viruses in wastewater and in wastewater-polluted environments. These studies have indicated good correlation between local viral outbreaks and high quantities of norovirus

Table 1

Methods used for wastewater concentration and SARS-CoV-2 RNA quantification. Gc: genome copies; MgV: mengovirus; PEDV: porcine epidemic diarrhoea virus; polyethylene glycol. *Preprint (not peer reviewed).

Region	Sampling dates	Wastewater type	Volume ml	Concentration	RT-(q)PCR target region	Process control recovery	SARS-CoV-2 detection rates	SARS-CoV-2 concentration gc/100 ml	Reference
Milan and Rome, Italy	03/02/2020–02/04/2020	Untreated wastewater	250	PEG/dextran precipitation of centrifuged supernatant	ORF1ab gene S gene	NA	12/12	NA (PCR detection)	[33]
Netherlands	05/02/2020–16/03/2020	Untreated wastewater	36–150	Centricon (Merck) ultrafiltration of centrifugated supernatant	N gene E gene	50% FRNA phage recovery	N1: 14/24 N2: 0/24 N3: 8/24 E: 5/24	NA	[7]*
Valencia, Spain	12/02/2020–14/04/2020	Untreated wastewater	200	Aluminium flocculation – beef extract precipitation	N gene	NA	12/15	10^4–10^5	[14]*
		Treated wastewater					0/9	0	
Southeast Queensland, Australia	24/02/2020–04/04/2020	Untreated wastewater	100–200	pH adjustment to ~4 and electronegative filtration	N gene	NA	N_Sarbecco: 1/9 NIID_2019-nCOV_N: 0/9	12	[6]
				Centricon (Merck) ultrafiltration of centrifugated supernatant			N_Sarbecco: 1/9 NIID_2019-nCOV_N: 0/9	1.9	
Wuchang Fangcang Hospital, China	26/02/2020–10/03/2020	Untreated wastewater	NA	PEG precipitation of centrifugated supernatant	ORF1 N gene	NA	0/4		[13]*
		Treated wastewater					7/9	$0.05–1.87 \times 10^4$	
Paris, France	05/03/2020–09/04/2020	Untreated wastewater	11	Ultracentrifugation	E gene	NA	23/23	10^3–10^6	[16]*
		Treated wastewater					6/8	10^2–10^4	
Paris, France	05/03/2020–23/04/2020	Untreated wastewater	11	Ultracentrifugation	E gene RdRp gene	NA	100%	10^3–10^6	[17]*
Various locations, Israel	10/03/2020–21/04/2020	Untreated wastewater	250–1000	Primary: PEG or Alum precipitation of centrifuged supernatant. Secondary: Amicon ultrafiltration	E gene	NA	10/26	NA	[8]*
Murcia, Spain	12/03/2020–14/04/2020	Untreated wastewater	200	Aluminium flocculation – beef extract precipitation	N gene	PEDV: 10.90 ± 3.54% MgV: 10.85 ± 2.11%	N1: 21/42 N2: 23/42 N3: 27/42	N1: 1.4×10^4 N2: 3.4×10^4 N3: 3.1×10^4	[15]
		Treated wastewater				PEDV: 3.29 ± 1.58% MgV:6.19 ± 1.00%	Secondary: 2/18 Tertiary: 0/12	$<2.5 \times 10^4$	
Massachusetts, USA	18/03/2020–25/03/2020	Untreated wastewater	NA	PEG precipitation of filtered sample	N gene	NA	N1: 4/6 N2: 1/6 N3: 4/6	N1: $10^3 – 2 \times 10^4$ N2: $3 \times 10^3 – 10^4$ N3: 10^3–10^4	[12]*

New Haven, Connecticut, USA	19/03/2020–01/05/2020	Primary sludge	2.5	Direct RNA extraction	N gene	NA	44/44	1.7×10^5 – 4.6×10^7	[18]*
Bozeman, Montana, USA	23/03/2020–08/04/2020	Untreated wastewater	500	Coming Spin-X ultrafiltration of filtered sample	N gene	NA	N1: 7/7 N2: 7/7	N1: 10^2–10^4 N2: 10^2 – 3×10^4	[10]*
Ourense, Spain	06/04/2020–21/04/2020	Untreated wastewater Treated wastewater Sludge	250	Amicon ultrafiltration of centrifugated supernatant Glycine/beef extract elution – centrifugation – filtration - PEG precipitation	N gene E gene RdRp gene	MS2 phage: 33.3 ± 15.6%	Influent: 5/5 Primary effluent: 1/4 Effluent: 0/5 14/34	NA	[11]*
Istambul, Turkey	21/04/2020–25/04/2020	Untreated wastewater	250	Amicon ultrafiltration OR PEG precipitation of centrifugated supernatant	RdRp gene	NA	7/9	10^2–10^5	[9]*

[34], hepatitis A and E viruses [35,36] and enterovirus D68 [36,37] in sewage. Although the presence of respiratory viruses in wastewater has arguably received less attention, several countries have detected SARS-CoV-2 in sewage (Table 1). No SARS-CoV-2 was reported in wastewater before the first cases [7]; however, there is some indication that SARS-CoV-2 was present in wastewater at Amersfoort, the Netherlands days before the first cases were reported [38]. When the temporal changes in SARS-CoV-2 titres were assessed, viral concentrations showed good correlations with the number of COVID-19 cases in the community [14,16,17]. Consequently, wastewater-based epidemiology may find future application as an early warning system for virus outbreaks, to monitor the progression of viral outbreaks, and in the provision of viral genomic data at the population scale.

Implications for the wider environment

Five studies have investigated viral titres in treated wastewater and three of those have found SARS-CoV-2 RNA in effluent with concentrations up to 10^4 gc/100 ml, suggesting 1−2 $\log_{10}$ removal during wastewater treatment [13,15,16]. Whether this poses a major risk to the wider environment remains unclear. However, recent reports suggest that SARS-CoV-2 can also infect and replicate in semiaquatic secondary animal vectors such as mink [39,40]. This offers the potential for animals close to wastewater outlets to readily come into contact with SARS-CoV-2 from which it would likely become endemic in the secondary host. This is most likely to occur from the discharge of untreated sewage or from poorly treated wastewater close to watercourses (e.g. septic tanks). Considering the high concentrations of SARS-CoV-2 RNA in wastewater (up to 10^7 genome copies/l) [16], some virus particles may be intact and infectious in sewage and hence viral infectivity in treated and untreated wastewater should be investigated. However, even if sewage contains infectious viruses, the likelihood of humans contracting SARS-CoV-2 from bathing waters or shellfish is likely to be extremely low, given the low stability of the virus in water and the large dilution of wastewater in inland waterbodies or coastal regions.

Public health and policy

Although the global clinical surveillance for COVID-19 has been established, there are a number of cases of asymptomatic individuals and those with very mild symptoms would not be identified and contacts not traced potentially missing an estimated 80% of actual transmission [41]. Monitoring SARS-CoV-2 in wastewater is therefore ideally suited to describe the spatial and temporal trends in disease incidence. Wastewater-based epidemiology may be useful to identify emerging and re-emerging pathogens in a community and may serve as an early warning system, which would be useful for public health mitigation [42,43]. However,

translating the viral titres from wastewater into the actual number of cases within a community is highly challenging, if not impossible. This type of calculation relies on many assumptions, which still remain poorly quantified (e.g. the amount and dynamics of viral shedding in faeces, viral persistence in the sewer network, variation in wastewater flow due to climate, etc). In addition, while suited to large urban communities (i.e. populations >10,000), the approach is less well suited from an economic and logistical perspective to disparate rural communities which may have hundreds of small water treatment facilities.

Although wastewater surveillance of SARS-CoV-2 provides a powerful tool to evaluate disease incidence at the community level, it is clear that they also need to be integrated into other public health initiatives [e.g. campaign-based and randomised testing of individuals (i.e. presence of pathogen or antibodies), clinical case reporting, and mobile-based contact-tracking and self-reporting systems [44]. This represents a significant challenge considering the poor integration of the environmental and clinical science communities. It may also require a harmonization of approaches (e.g. viral sequencing platforms and databases to match SARS-CoV-2 lineages detected in clinical and wastewater samples). It is also important to consider how best to ethically and legally balance public health with civil liberties when handling this information [45]. One of the benefits of wastewater, however, is that it has limited sociological bias with few if any ethical issues.

Conclusions and recommendations

Current data on SARS-CoV-2 and other viruses suggest that wastewater-based epidemiology is a viable addition to the assessment and mitigation of viral outbreaks. Easy and straightforward methods are available for the concentration of wastewater samples for viral detection, however, the use of process controls (e.g. spiking the sample with an animal virus with structure similar to the target pathogen before concentration) is recommended. The widely used q(RT-)PCR approach enables rapid and strain level RNA/DNA quantification, however, the primers and probes should be chosen carefully. Although the infectivity state of the target virus is not relevant for epidemiological surveillance, the survival of SARS-CoV-2 in sewage, during wastewater treatment and in the aquatic environment should be investigated to assess health risks. Targeted and untargeted sequencing of wastewater viruses has the potential to track the spread of specific sequence variants and identify mutations that could affect detection in clinical settings.

Conflict of interest statement

Nothing declared.

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References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Who: *Coronavirus disease 2019 (COVID-19) Situation report*. World Heal Organ; 2020.

2. Adriaenssens EM, Farkas K, Harrison C, Jones DL, Allison HE, McCarthy AJ, Jones DL: **Viromic analysis of wastewater input to a river catchment reveals a diverse assemblage of RNA viruses**. *mSystems* 2018, **3**. e00025-18.

3. Radin D: **New trends in food-and waterborne viral outbreaks**. *Arch Biol Sci* 2014, **66**:1–9.

4. Haramoto E, Kitajima M, Hata A, Torrey JR, Masago Y, Sano D, Katayama H: **A review on recent progress in the detection methods and prevalence of human enteric viruses in water**. *Water Res* 2018, **135**:168–186.

5. Bofill-Mas S, Rusiñol M: **Recent trends on methods for the concentration of viruses from water samples**. *Curr Opin Environ Sci Heal* 2020, **16**:7–13.

6. * Ahmed W, Angel N, Edson J, Bibby K, Brien JWO, Choi PM, Kitajima M, Simpson L, Li J, Tscharke B, *et al.*: **First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community**. *Sci Total Environ* 2020, https://doi.org/10.1016/j.scitotenv.2020.138764.
This is the first peer reviewed study on the presence of SARS-CoV-2 in wastewater using qRT-PCR detection and Sanger and Illumina sequencing for verification.

7. Medema G, Heijnen L, Elsinga G, Italiaander R: **Presence of SARS-Coronavirus-2 in sewage**. *MedRxiv* 2020, https://doi.org/10.1101/2020.03.29.20045880.

8. Bar Or I, Yaniv K, Shagan M, Ozer E, Erster O, Mendelson E, Mannasse B, Shirazi R, Kramarsky-Winter E, Nir O, *et al.*: **Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance**. *medRxiv* 2020, https://doi.org/10.1101/2020.04.26.20073569.

9. Kocamemi BA, Kurt H, Hacioglu S, Yarali C, Saatci AM, Pakdemirli B: **SARS-CoV-2 detection in istanbul wastewater treatment plant sludges**. *medRxiv* 2020, https://doi.org/10.1101/2020.05.12.20099358.

10. Nemudryi A, Nemudraia A, Surya K, Wiegand T, Buyukyoruk M, Wilkinson R, Wiedenheft B: **Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater**. *medRxiv* 2020, https://doi.org/10.1101/2020.04.15.20066746.

11. Balboa S, Mauricio-Iglesias M, Rodríguez S, Martínez-Lamas L, Vasallo FJ, Regueiro B, Lema JM: **The fate of SARS-CoV-2 in wastewater treatment plants points out the sludge line as a suitable spot for incidence monitoring**. *medRxiv* 2020.05.25, 20112706, https://doi.org/10.1101/2020.05.25.20112706.

12. Wu F, Xiao A, Zhang J, Gu X, Lee WL, Kauffman K, Hanage W, Matus M, Ghaeli N, Endo N: **SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases**. *medRxiv* 2020.

13. Zhang D, Ling H, Huang X, Li J, Li W, Yi C, Zhang T, Jiang Y, He Y, Deng S, *et al.*: **Potential spreading risks and disinfection challenges of medical wastewater by the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA in septic tanks of fangcang hospital**. *medRxiv* 2020, https://doi.org/10.1101/2020.04.28.20083832.

14. Randazzo W, Cuevas-Ferrando E, Sanjuan R, Domingo-Calap P, Sanchez G: **Metropolitan wastewater analysis for COVID-19 epidemiological surveillance**. *medRxiv* 2020.04.23, 20076679, https://doi.org/10.1101/2020.04.23.20076679.

15. ** Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G: **SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area**. *Water Res* 2020, **181**, 115942, https://doi.org/10.1016/j.watres.2020.115942.
This is the first peer-reviewed publication on the temporal surveillance of SARS-CoV-2 in wastewater. This is the first study which assessed sample process performance using process control viruses. The investigation found SARS-CoV-2 RNA in secondary treated wastewater but not in tertiary treated effluent.

16. Wurtzer S, Marechal V, Jm M, Moulin L, Université S, Metis UMR, Atelier Z: **Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases**. *MedRxiv* 2020, https://doi.org/10.1101/2020.04.12.20062679.

17. Wurtzer S, Marechal V, Mouchel J-M, Maday Y, Teyssou R, Richard E, Almayrac JL, Moulin L: **Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters**. *medRxiv* 2020, https://doi.org/10.1101/2020.04.12.20062679.

18. Peccia J, Zulli A, Brackney DE, Grubaugh ND, Kaplan EH, Casanovas-Massana A, Ko AI, Malik AA, Wang D, Wang M, *et al.*: **SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics**. *medRxiv* 2020, https://doi.org/10.1101/2020.05.19.20105999.

19. Farkas K, Mannion F, Hillary LS, Malham SK, Walker DI: **Emerging technologies for the rapid detection of enteric viruses in the aquatic environment**. *Curr Opin Environ Sci Heal* 2020, **16**:1–6.

20. Jiang Y, Fang L, Shi X, Zhang H, Li Y, Lin Y, Qiu Y, Chen Q, Li H, Zhou L, *et al.*: **Simultaneous detection of five enteric viruses associated with gastroenteritis by use of a PCR assay: a single real-time multiplex reaction and its clinical application**. *J Clin Microbiol* 2014, **52**:1266–1268.

21. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, Petrone ME, Landry M-L, Foxman EF, Grubaugh ND: **Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR assays**. *medRxiv* 2020.03.30, 20048108, https://doi.org/10.1101/2020.03.30.20048108.

22. Corman V, Bleicker T, Brünink S, Drosten C, Zambon M, World Health Organization: *Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR*. Geneva: World Health Organization; 2020. January, 13.

23. Chan JF-W, Yip CC-Y, To KK-W, Tang TH-C, Wong SC-Y, Leung K-H, Fung AY-F, Ng AC-K, Zou Z, Tsoi H-W: **Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens**. *J Clin Microbiol* 2020.

24. * Nalla AK, Casto AM, Huang M-LW, Perchetti GA, Sampoleo R, Shrestha L, Wei Y, Zhu H, Jerome KR, Greninger AL: **Comparative performance of SARS-CoV-2 detection assays using seven different primer/probe sets and one assay kit**. *J Clin Microbiol* 2020.
This study performs a comparative assessment of the usefulness of qRT-PCR assays commonly used for the detection of SARS-CoV-2.

25. Mao K, Zhang H, Yang Z: **Can a paper-based device trace COVID-19 sources with wastewater-based epidemiology?** *Environ Sci Technol* 2020, **54**:3733–3735, https://doi.org/10.1021/acs.est.0c01174.

26. Ishii S, Kitamura G, Segawa T, Kobayashi A, Miura T, Sano D, Okabe S: **Microfluidic quantitative PCR for simultaneous quantification of multiple viruses in environmental water samples**. *Appl Environ Microbiol* 2014, **80**:7505–7511.

27. Xiao F, Sun J, Xu Y, Li F, Huang X, Li H, Al E: **Infectious SARS-CoV-2 in feces of patient with severe COVID-19**. *Emerg Infect Dis* 2020, **26**, https://doi.org/10.3201/eid2608.200681.

28. Sun J, Zhu A, Li H, Zheng K, Zhuang Z, Chen Z, Shi Y, Zhang Z, Chen S, Liu X: **Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient**. *Emerg Microb Infect* 2020.

29. Bibby K, Crank K, Greaves J, Li X, Wu Z, Hamza IA, Stachler E: **Metagenomics and the development of viral water quality tools**. *npj Clean Water* 2019, **2**:1–13.

30. St Hilaire BG, Durand NC, Mitra N, Pulido SG, Mahajan R, Blackburn A, Colaric ZL, Theisen JWM, Weisz D, Dudchenko O: **A rapid, low cost, and highly sensitive SARS-CoV-2 diagnostic based on whole genome sequencing**. *bioRxiv* 2020.

31. Bisseux M, Didier D, Audrey M, Christine A, Hélène PL, Jean-Luc B, Cécile H: **Monitoring of enterovirus diversity in wastewater by ultra-deep sequencing: an effective complementary tool for clinical enterovirus surveillance**. *Water Res* 2020:169.

32. Lun JH, Crosbie ND, White PA: **Genetic diversity and quantification of human mastadenoviruses in wastewater from Sydney and Melbourne, Australia**. *Sci Total Environ* 2019, **675**:305–312.

33. * La Rosa G, Iaconelli M, Mancini P, Bonanno Ferraro G, Veneri C, Bonadonna L, Lucentini L, Suffredini E: **First detection of SARS-CoV-2 in untreated wastewaters in Italy**. *Sci Total Environ* 2020, **736**:139652.
This study reported SARS-CoV-2 in wastewater in Milan only a few days after the first COVID-19 cases were confirmed. Sequencing of SARS-CoV-2 isolated from wastewater showed 100% identity with the SARS-CoV-2 genome isolated from the first COVID-19 case in Italy.

34. Farkas K, Cooper DM, McDonald JE, Malham SK, de Rougemont A, Jones DL, de Rougemont A, Jones DL, de Rougemont A, Jones DL: **Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters**. *Sci Total Environ* 2018, **634**:1174–1183.

35. Miura T, Lhomme S, Le Saux JC, Le Mehaute P, Guillois Y, Couturier E, Izopet J, Abranavel F, Le Guyader FS: **Detection of hepatitis E virus in sewage after an outbreak on a French island**. *Food Environ Virol* 2016, **8**:194–199.

36. Bisseux M, Colombet J, Mirand A, Roque-Afonso AM, Abravanel F, Izopet J, Archimbaud C, Peigue-Lafeuille H, Debroas D, Bailly JL, *et al.*: **Monitoring human enteric viruses in wastewater and relevance to infections encountered in the clinical setting: a one-year experiment in central France, 2014 to 2015**. *Euro Surveill* 2018, **23**:1–11.

37. Majumdar M, Wilton T, Hajarha Y, Klapsa D, Martin J: **Detection of Enterovirus D68 in wastewater samples from the United Kingdom during outbreaks reported globally between 2015 and 2018**. *bioRxiv* 2019.

38. Mallapaty S: **How sewage could reveal true scale of coronavirus outbreak**. *Nature* 2020, **580**:176–177.

39. Kim Y-I, Kim S-G, Kim S-M, Kim E-H, Park S-J, Yu K-M, Chang J-H, Kim EJ, Lee S, Casel MAB, *et al.*: **Infection and rapid transmission of SARS-CoV-2 in ferrets**. *Cell Host Microbe* 2020, **27**:704–709.e2.

40. Oreshkova N, Molenaar R-J, Vreman S, Harders F, Munnink BBO, Hakze R, Gerhards N, Tolsma P, Bouwstra R, Sikkema R, *et al.*: **SARS-CoV2 infection in farmed mink, Netherlands, April 2020**. *bioRxiv* 2020, https://doi.org/10.1101/2020.05.18.101493.

41. Larsen D, Dinero R, Asiago-Reddy E, Green H, Lane S, Shaw A, Zeng T, Kmush B: *A review of infectious disease surveillance to inform public health action against the novel coronavirus SARS-CoV-2*. 2020.

42. ** Sims N, Kasprzyk-Hordern B: **Future perspectives of wastewater-based epidemiology: monitoring infectious**

disease spread and resistance to the community level. *Environ Int* 2020, **139**:105689.
This is a very thorough review on the aspects of wastewater-based epidemiology, highlighting the different aspects of surveillance, the benefits and limitations of wastewater surveillance and an overview of pathogens and biomarkers in wastewater.

43. Xagoraraki I, O'Brien E: **Wastewater-based epidemiology for early detection of viral outbreaks**. In *Women in water quality*. Edited by O'Bannon DJ, E. Lansing, MI: Michigan State University; 2020:75–97. UNESCO.

44. Boulos MNK, Geraghty EM: **Geographical tracking and mapping of coronavirus disease COVID-19/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic and associated events around the world: how 21st century GIS technologies are supporting the global fight against outbr**. *Int J Health Geogr* 2020, **19**:8.

45. Gostin LO, Friedman EA, Wetter SA: **Responding to COVID-19: how to navigate a public health emergency legally and ethically**. *Hastings Cent Rep* 2020, **50**:8–12.